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STUDIES ON THE BIODISPOSITION OF ORGANOPHOSPHATES IN MICE

Annual Summary Report

Billy R. Martin, Ph.D.

August 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701-5012

Contract Number DAMD17-82-C-2212

Virginia Commonwealth University  
Richmond, Virginia 23298

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85 3 27 060

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM	
1. REPORT NUMBER	2. GOVT ACCESSION NO. <b>AD-B090 890</b>	3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle)  Studies on the Biodisposition of Organophosphates in Mice		5. TYPE OF REPORT & PERIOD COVERED Annual Summary Report Aug. 1, 1982-July 31, 1983	
		6. PERFORMING ORG. REPORT NUMBER	
7. AUTHOR(s)  Billy R. Martin, Ph.D.		8. CONTRACT OR GRANT NUMBER(s)  DAMD 17-82-C-2212	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Pharmacology & Toxicology Medical College of Virginia, Box 613 Virginia Commonwealth Univ., Richmond, VA 23298		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 6.27.34.A 3M162734A875 AE.113	
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Fort Detrick, Frederick, Maryland 21701-5012		12. REPORT DATE August, 1983	
		13. NUMBER OF PAGES 38	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report)  Unclassified	
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report) Distribution limited to US Government agencies and their contractors; Administrative/Operational Use, December 18, 1984. Other requests for this document shall be referred to Commander, US Army Medical Research and Development Command, ATTN: SGRD-RMS, Fort Detrick, Frederick, Maryland 21701-5012.			
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)			
18. SUPPLEMENTARY NOTES			
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) diisopropylfluorophosphate, tissue disposition, anticholinesterase activity.			
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The biodisposition of radioactivity was studied in mice following the i.v. administration of $^3\text{H}$ -DFP (1 mg/kg). $^3\text{H}$ -DFP penetrated tissues rapidly so that within 1 min of drug administration, maximal levels of radioactivity were found in brain, lung, heart and kidney. The peak levels of radioacti- vity in liver, diaphragm and fat occurred at 5 min. Tissue levels remained elevated for approximately 8 hr. The rank order of tissue concentrations			

## 20. ABSTRACT continued...

During this period of time was liver > plasma > kidney > lung > heart = diaphragm > brain = fat. Radioactivity declined appreciably by 3 days, and after 7 days the highest concentrations were found in liver and lung with somewhat lower concentrations in kidney. It may be that lung and liver are potential storage depots under certain circumstances. Radioactivity was readily extracted from brain homogenates (55% removed by ethyl acetate) 1 min after administration, but the amount that was nonextractable increased with time. A relatively small portion (13-24%) of the radioactivity was extractable from the other tissues at the early time points, but even these small percentages decreased with time. It appears that enzyme aging occurs slowly or to a limited degree in mice, but further experimentation will be necessary to establish the extent to which it occurs. Original  
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# SUMMARY

The biodisposition of radioactivity was studied in mice following the i.v. administration of  $^3\text{H}$ -DFP (1 mg/kg).  $^3\text{H}$ -DFP penetrated tissues rapidly so that within 1 min of drug administration, maximal levels of radioactivity in liver, diaphragm and fat occurred at 5 min. Tissue levels remained elevated for approximately 8 hr. The rank order of tissue concentrations during this period of time was liver > plasma > kidney > lung > heart = diaphragm > brain = fat. Radioactivity declined appreciably by 3 days, and after 7 days the highest concentrations were found in liver and lung with somewhat lower concentrations in kidney. It may be that lung and liver are potential storage depots under certain circumstances. Radioactivity was readily extracted from brain homogenates (55% removed by ethyl acetate) 1 min after administration, but the amount that was nonextractable increased with time. A relatively small portion (13-24%) of the radioactivity was extractable from the other tissues at the early time points, but even these small percentages decreased with time. It appears that enzyme aging occurs slowly or to a limited degree in mice, but further experimentation will be necessary to establish the extent to which it occurs.

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## FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

# TABLE OF CONTENTS

SUMMARY	Page 2
FOREWARD	3
I. INTRODUCTION	6
II. CHOLINESTERASE ACTIVITY	
A. Methods	6
B. Results and Discussion	7
1. Incubation time course for brain, diaphragm and plasma cholinesterase activity in mice and rats	7
2. Effect of DFP on cholinesterase activity	7
3. Acetylcholinesterase activity in mouse brain areas	11
III. BIODISPOSITIONAL STUDIES OF $^3\text{H}$ -DFP IN MICE	
A. Methods	11
B. Results and Discussion	13
1. Tissue levels of total radioactivity	13
2. Long-term biodisposition of radioactivity	23
3. Extractions of unbound radioactivity from tissue homogenates	23
C. Conclusions	33
IV. LITERATURE CITED	35
V. FIGURES AND TABLES:	
A. Figures	
1. Figure 1: Time Course for Hydrolysis of $^{14}\text{C}$ ACh by Rat Brain, Plasma and Diaphragm	8
2. Figure 2: Time Course for Hydrolysis of $^{14}\text{C}$ ACh by Mouse	9
3. Figure 3: Time Course of Radioactivity in Plasma After i.v. Injection of $^3\text{H}$ -DFP	14
4. Figure 4: Time Course of Radioactivity in Brain After i.v. Injection of $^3\text{H}$ -DFP	15
5. Figure 5: Time Course of Radioactivity in Lung After i.v. Injection of $^3\text{H}$ -DFP	16
6. Figure 6: Time Course of Radioactivity in Diaphragm After i.v. Injection of $^3\text{H}$ -DFP	18
7. Figure 7: Time Course of Radioactivity in Liver After i.v. Injection of $^3\text{H}$ -DFP	19
8. Figure 8: Time Course of Radioactivity in Fat After i.v. Injection of $^3\text{H}$ -DFP	20
9. Figure 9: Time Course of Radioactivity in Kidney After i.v. Injection of $^3\text{H}$ -DFP	21
10. Figure 10: Time Course of Radioactivity in Heart After i.v. Injection of $^3\text{H}$ -DFP	22
11. Figure 11: Long-Term Time Course of Radioactivity in Plasma After i.v. Injection of $^3\text{H}$ -DFP	24
12. Figure 12: Long-Term Time Course of Radioactivity in Brain After i.v. Injection of $^3\text{H}$ -DFP	25

	<u>Page</u>
13. Figure 13: Long-Term Time Course of Radioactivity in Lung After i.v. Injection of $^3\text{H}$ -DFP	26
14. Figure 14: Long-Term Time Course of Radioactivity in Diaphragm After i.v. Injection of $^3\text{H}$ -DFP	27
15. Figure 15: Long-Term Time Course of Radioactivity in Liver After i.v. Injection of $^3\text{H}$ -DFP	28
16. Figure 16: Long-Term Time Course of Radioactivity in Fat After i.v. Injection of $^3\text{H}$ -DFP	29
17. Figure 17: Long-Term Time Course of Radioactivity in Kidney After i.v. Injection of $^3\text{H}$ -DFP	30
18. Figure 18: Long-Term Time Course of Radioactivity in Heart After i.v. Injection of $^3\text{H}$ -DFP	31
19. Figure 19: Fate of $^3\text{H}$ -DFP <u>In Vivo</u>	32

B. Tables

1. Table 1: Acetylcholinesterase Activity in Rats and Mice Treated with DFP	10
2. Table 2: Acetylcholinesterase Activity in Mouse Brain Areas	11
3. Table 3: Extraction of $^3\text{H}$ -DFP from Mouse Brain Homogenates	12
4. Table 4: Extraction of Tissue Radioactivity from Animals Treated with $^3\text{H}$ -DFP	33

## I. INTRODUCTION

The objective of the research as outlined in our current contract is to study the biodisposition of organophosphates, namely, diisopropyl-fluorophosphate (DFP), soman, sarin and tabun, in mice. Our aims are to identify site(s) of action of these organophosphates and to formulate the means by which we may alter the biodisposition of these agents to ameliorate their toxicity. The relative lack of published information regarding the biodisposition of these organophosphates has prompted us to investigate their tissue disposition after different routes of administration (those most relevant to human exposure), after acute and chronic administration and in the presence of oximes. A correlation between cholinesterase activity and tissue levels of organophosphate will also be established in an attempt to delineate central and peripheral actions. In addition, acetylcholine levels in brain areas will be correlated with the disposition of the organophosphates.

This report deals with cholinesterase activity in mice and rats, preliminary results in development of quantitative assays for measuring unreacted organophosphate in tissues and time course of tissue levels of  $^3\text{H}$ -DFP after acute treatment. These experiments have enabled us to establish procedures to be used with the organophosphates soman, sarin and tabun as well as provide interesting insights into the biodisposition of DFP.

## II. CHOLINESTERASE ACTIVITY

### A. Methods

The method of Siakotos et al. (1) was adopted for measuring cholinesterase activity with acetylcholine as the substrate. No attempts were made to distinguish pseudocholinesterase and acetylcholinesterase activity.  $^{14}\text{C}$ -Acetylcholine chloride (Amersham) is diluted with acetylcholine chloride to make a  $3 \times 10^{-3}$  M solution containing 1  $\mu\text{Ci}/\text{m}$ . The resin-dioxane mixture and buffers are prepared as described by Siakotos et al. (1). Blood from the cervical wound of male Sprague-Dawley rats or CD-1 mice (Dominion Laboratories, Dublin, VA) is collected in plastic heparinized tubes which are centrifuged to separate plasma and red blood cells. The plasma is diluted 10-fold with distilled water and then assayed for enzyme activity. Whole brains and diaphragm are homogenized in 9 volumes of 0.4 M sucrose and then diluted 10-fold with phosphate buffer. Brains are dissected as described by Glowinski and Iversen (2) and each brain area is homogenized in 100 volumes of phosphate-buffer sucrose (0.04 M).

To measure cholinesterase activity, 100  $\mu\text{l}$  of buffer and 100  $\mu\text{l}$  of the  $^{14}\text{C}$ -ACh solution are added to tubes followed by 100  $\mu\text{l}$  of sample. Brain and plasma samples are incubated for 10 min and diaphragm is incubated for 30 min, after which time the reaction is stopped by the addition of 5 ml of a resin-dioxane solution. Additional dioxane (5 ml) is added, the contents mixed, and the samples centrifuged at 3,000

rpm for 2 min. A 5 ml aliquot of the dioxane supernatant is pipetted off and added to TPP-TX for liquid scintillation spectrometry.

DFP was purchased from Sigma Chemical Company and  $^3\text{H}$ -DFP was obtained from New England Nuclear in propylene glycol. Both compounds are stored in a dessicator at  $2^\circ\text{C}$ . The DFP is prepared for injection by pipetting DFP into a tared weighing vial which is then weighed. The appropriate volume of saline is added and the sample is used within 2 hr of preparation.

## B. Results and Discussion

### 1. Incubation time course for brain, diaphragm and plasma cholinesterase activity in mice and rats.

Experiments were carried out to determine the incubation time during which enzymatic hydrolysis of ACh is linear. Tissues from rats ( $N=2$  for each time point) were examined first for purposes of comparison with Siakotos *et al.* (1). The results ( $N=5$ ) presented in Figure 1 are similar to those of Siakotos *et al.* (1) in that hydrolysis by brain cholinesterase was linear up to 10 min before reaching a plateau at 30 min. The activity in rat plasma was linear up to 60 min, which was slightly different from that reported by Siakotos *et al.* (1) who found a slight decrease in activity between 30 and 60 min. Hydrolysis in diaphragms, a tissue which Siakotos *et al.* did not study, was also linear up to 60 min. Based upon these data, we chose a 10-min incubation for analysis of brain and plasma cholinesterase activity and a 30-min incubation for diaphragm cholinesterase activity. After finding that our results with rats were in agreement with those of Siakotos *et al.* (1), we investigated the incubation time courses for brain, diaphragm and plasma from mice, the species to be used throughout our studies. Siakotos *et al.* (1) did not present any time course data for tissues from mice. The results in Figure 2 show considerable agreement between mice ( $N=5$ ) and rat ( $N=5$ ) cholinesterase activity. Brain and plasma cholinesterase activity is linear for the first 20 min, whereas the diaphragm is linear for at least 60 min. Therefore, 10-min incubations were chosen for mouse plasma and brain and 30-min incubations for diaphragm. The major difference between mouse and rat cholinesterase is the higher rate of hydrolysis in the mouse tissues.

### 2. Effect of DFP on cholinesterase activity.

Mice and rats were injected i.v. with either DFP or saline and decapitated 15 min later. AChE activities in plasma and brain and diaphragm homogenates are presented in Table 1. The AChE activity in rat brains from the saline-treated animals is similar to that reported by Siakotos *et al.* (1), while the plasma levels are somewhat lower than their values of 10 nmoles/mg protein/min. The level of AChE activity in the diaphragm from saline-treated rats is in good agreement with the 6 nmoles/mg protein/min reported by Sterri *et al.* (3). As expected, DFP produced a dramatic dose-related inhibition of AChE activity in all tissues. The AChE activity in brain homogenates and plasma from saline-

Figure 1

Time Course for Hydrolysis of  $^{14}\text{C}$ -ACh by Rat  
Brain, Plasma and Diaphragm. Mean of six replicates.

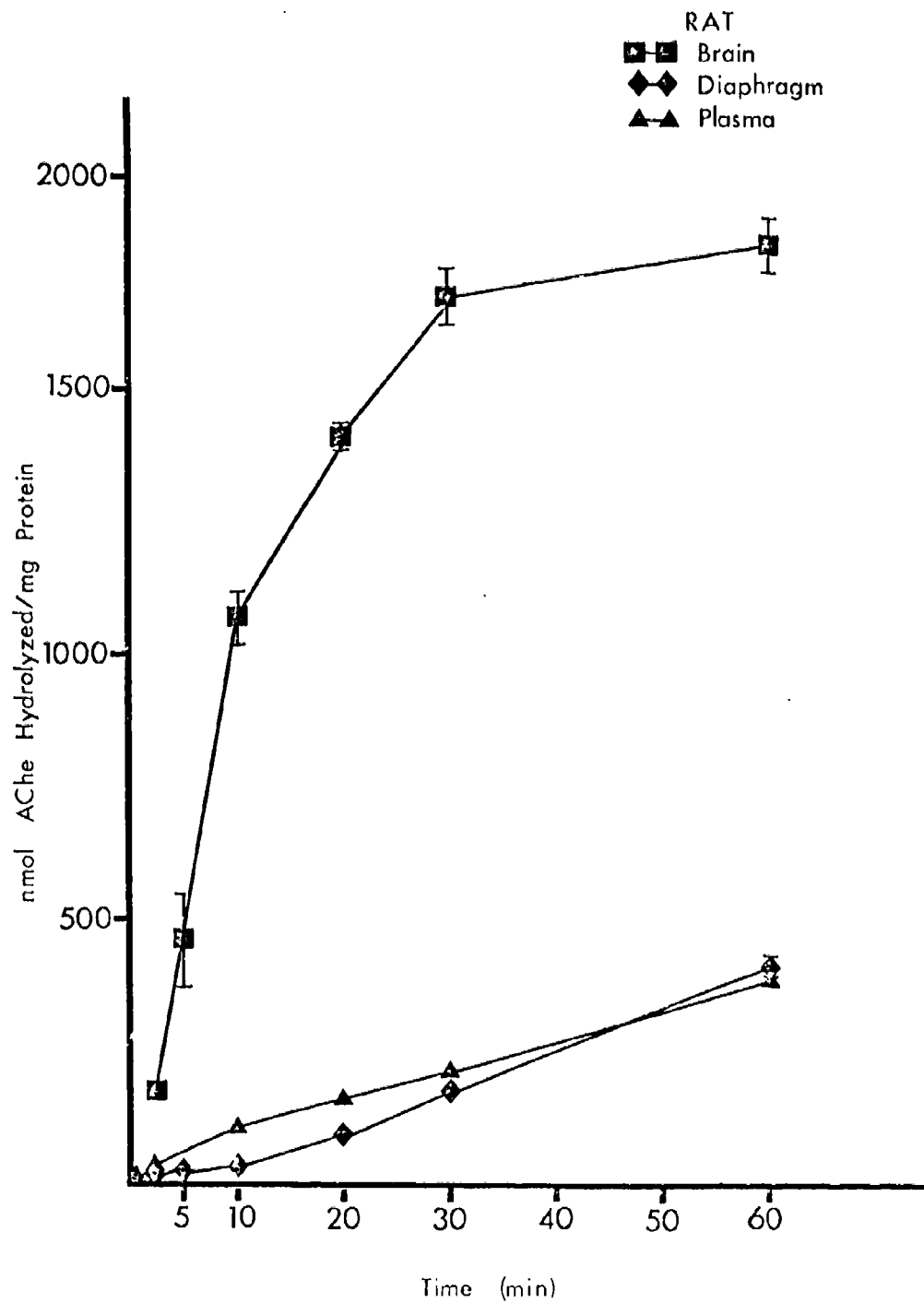


Figure 2

Time Course for Hydrolysis of  $^{14}\text{C}$ -ACh by Mouse Brain Plasma and Diaphragm.  
Tissues from animals were pooled and assayed in triplicate.

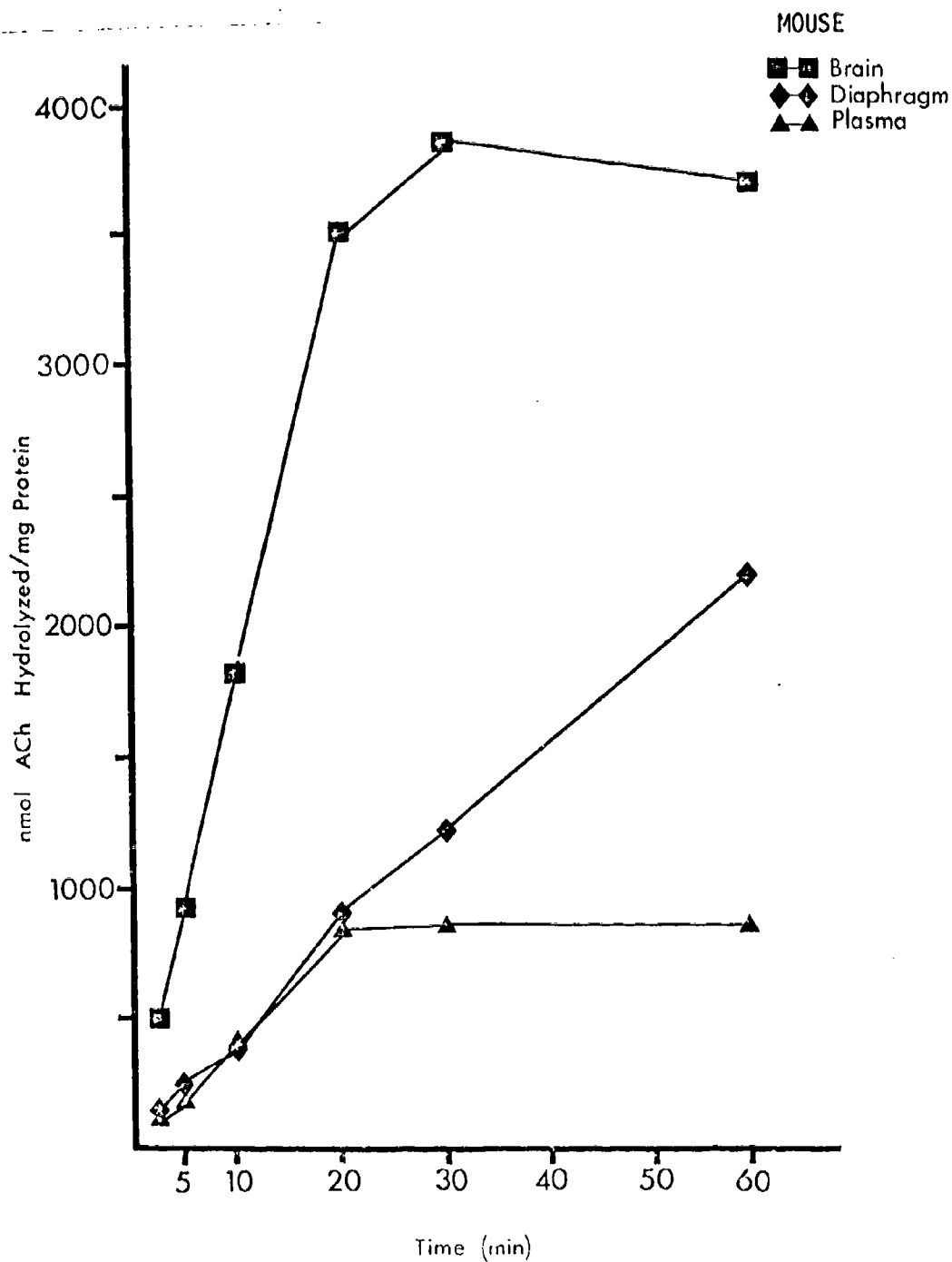


TABLE 1

## Acetylcholinesterase Activity in Rats and Mice Treated with DFP

<u>Treatment</u>	<u>Dose<sup>A</sup></u>	<u>N</u>	<u>Tissue</u>	<u>AChE Activity<sup>B</sup></u>	<u>% Inhibition</u>
RATS					
Saline	-	6	Brain	78 ± 7	-
DFP	0.5	6	Brain	33 ± 3	54
DFP	1.0	6	Brain	5 ± 0	94
Saline	-	10	Plasma	3.9 ± 0.3	-
DFP	0.5	4	Plasma	1.2 ± 0.2	70
DFP	1.0	6	Plasma	0.7 ± 0.1	82
Saline	-	10	Diaphragm	7.0 ± 1.1	-
DFP	0.5	6	Diaphragm	5.5 ± 1.3	22
DFP	1.0	6	Diaphragm	0.3 ± 0.1	95
MICE					
Saline	-	6	Brain	318 ± 81	-
DFP	1.0	6	Brain	78 ± 15	75
DFP	2.0	6	Brain	24 ± 5	92
Saline	-	6	Plasma	17.1 ± 1.2	-
DFP	1.0	6	Plasma	3.2 ± 0.5	82
DFP	2.0	6	Plasma	3.5 ± 0.3	76
Saline	-	6	Diaphragm	7.7 ± 2.1	-
DFP	1.0	6	Diaphragm	4.5 ± 1.5	38
DFP	2.0	6	Diaphragm	0 ± 0	100

<sup>A</sup> mg/kg

<sup>B</sup> nmole ACh hydrolyzed/mg protein/min.

treated mice was found to be considerably greater than that in rats; however, these values appear to be anomalous in that subsequent experiments with mice produced lower AChE values. However these data are presented here to demonstrate the fact that DFP inhibition of cholinesterase activity can be readily measured. The activity in diaphragm was comparable in both species in all experiments. DFP treatment in mice produced the expected decrease in AChE activity, although doses somewhat higher than those used in rats were required.

### 3. Acetylcholinesterase activity in mouse brain areas.

One of the objectives of this research is to correlate the biodisposition of organophosphates to cholinesterase activity and acetylcholine levels in brain areas. The results in Table 2 show the AChE activity in brain areas of untreated mice. Future experiments will be carried out with brain areas from mice treated with organophosphates.

TABLE 2

#### Acetylcholinesterase Activity in Mouse Brain Areas

<u>Brain Area</u>	<u>nmole ACh Hydrolyzed/mg Protein/min<sup>A</sup></u>
Corpus striatum	203 ± 30
Hippocampus	146 ± 22
Hypothalamus	141 ± 22
Midbrain	121 ± 12
Cortex	94 ± 14
Medulla/pons	82 ± 15
Cerebellum	36 ± 5
Whole Brain	105 ± 28

<sup>A</sup> Mean ± S.E. of N=8.

### III. BIODISPOSITIONAL STUDIES OF <sup>3</sup>H-DFP IN MICE

#### A. Methods

<sup>3</sup>H-DFP was added to DFP, which was then diluted appropriately with saline to provide a dose of 140  $\mu$ Ci/mg/kg. The stability of the <sup>3</sup>H-DFP was determined by analyzing the sample by thin-layer chromatography (TLC) at 30-min intervals during the experiment. TLC was carried out with silica gel plates (5x10 cm) which were developed in ethyl acetate. One-cm bands of the silica gel are removed for scintillation counting. DFP was found to have an RF of 0.75. TLC analysis of the sample

immediately after preparation revealed that 90% of the radioactivity corresponded to  $^3\text{H}$ -DFP and only 3% remained at the origin. After all of the animals had been injected (2.5 hr later), 88% radioactivity corresponded to  $^3\text{H}$ -DFP and only 5% remained at the origin.

The  $^3\text{H}$ -DFP (1 mg/kg) was administered i.v. over a 6-second span and five mice were decapitated either immediately, or after 15 sec; 1, 5, 15, 30 min, 1, 2, 3, 4, 8 hr, 1, 3, or 7 days. Blood from the cervical wound was collected in heparinized tubes and centrifuged to provide plasma. Brain, liver, diaphragm, heart, kidney, lung and fat were homogenized in 5 volumes of phosphate buffer (pH 7.4) containing 10% sucrose (0.4 M). Aliquots of tissue homogenates (250  $\mu\text{l}$ ) and plasma (50  $\mu\text{l}$ ) were solubilized with NCS reagent (Amersham) so that radioactivity could be quantitated by liquid scintillation spectrometry. Quench was corrected by using external standardization.

In order to extract  $^3\text{H}$ -DFP from tissues, preliminary experiments were carried out to establish the solvent of choice. Previously, chloroform has been used to remove organophosphates from blood (4), but unfortunately, it is unsuitable for scintillation counting due to the extreme quench it produces. To 3 ml of brain homogenates (10 mg tissue/ml) was added 2  $\mu\text{l}$  of  $^3\text{H}$ -DFP in ethyl acetate and the sample was mixed well. The pH was adjusted to either 4.0 or 6.2. Then 5 ml of either ethyl acetate, chloroform, diethyl ether or hexane was added to triplicate tubes, and the samples were shaken for 15 min and then centrifuged at 3,000 g for 10 min. Five ml of each solvent, except chloroform, was removed and added to scintillation fluid for quantitation of radioactivity. The chloroform aliquot was evaporated to dryness under a stream of nitrogen, scintillation fluid was added, and the sample was analyzed by scintillation spectrometry. Aliquots of the aqueous remainders were solubilized with NCS reagent and counted for radioactivity. The results in Table 3 show that ethyl acetate, hexane and diethyl ether effectively removed the  $^3\text{H}$ -DFP at both pH's. Very little of the radioactivity was found in the chloroform extract, which was probably due to loss during evaporation. Therefore, ethyl acetate was chosen for routine extractions, which were carried out at pH 6.2.

TABLE 3  
Extraction of  $^3\text{H}$ -DFP From Mouse Brain Homogenates

Solvent	Percent $^3\text{H}$ -DFP Extracted		Percent $^3\text{H}$ -DFP Remaining	
	pH 4.0	pH 6.2	pH 4.0	pH 6.2
Ethyl acetate	88	91	6	6
Chloroform	6	18	-	-
Hexane	83	84	16	16
Diethyl ether	88	88	15	16

In order to determine the quantity of radioactivity that was ethyl acetate extractable, mice were injected i.v. with  $^3\text{H}$ -DFP (100  $\mu\text{Ci}/\text{mg}$ ) at a dose of 1 mg/kg. Five mice were decapitated either 1 min, 30 min, 4 hr or 3 days later. Organs were quickly removed, weighed and placed in 2 ml of phosphate buffer (pH 7.4). Plasma (250  $\mu\text{l}$ ) was added to 2 ml of buffer. Ethyl acetate (10 ml) was added immediately and the samples were homogenized with a polytron for 1 min and then centrifuged for 10 min at 1,000  $\times$  g. Five ml of the ethyl acetate was removed for scintillation counting and 250  $\mu\text{l}$  of the aqueous remainder was solubilized for the same purpose.

## B. Results and Discussion

### 1. Tissue levels of total radioactivity.

The levels of total radioactivity in tissues up to 8 hr after administration of  $^3\text{H}$ -DFP (1 mg/kg, i.v.) are presented in Figures 3-10. The plasma levels of radioactivity declined in a biphasic fashion, as depicted in Figure 3 (data will be evaluated by pharmacokinetic analysis). The initial decline in radioactivity (the  $\alpha$  phase) that occurs during the first 30 min demonstrates the rapid transfer of  $^3\text{H}$ -DFP to tissues. This rapid entry into tissue is even more evident from plasma levels of radioactivity immediately after 15 sec after infusion (data not presented in graphs). Immediately after infusion, there were 8.2 ng-equivalents of  $^3\text{H}$ -DFP per  $\mu\text{l}$  plasma or 26% of the injected dose in the total plasma volume. Radioactivity associated with erythrocytes was not quantitated. By 15 sec, the concentration of  $^3\text{H}$ -DFP had fallen to 2.6 ng/ $\mu\text{l}$  plasma, or 8% of the injected dose in the total plasma volume. A 3-fold drop in plasma radioactivity during a 15-sec interval as well as the high tissue levels found at 1 min (Figures 4-10) clearly demonstrate the ease with which  $^3\text{H}$ -DFP penetrates tissues. DFP's rapid entry into tissue has not been reported previously but is similar to that recently reported for parathion, a thiophosphate (5). The slow decline in radioactivity between 30 min and 8 hr after  $^3\text{H}$ -DFP administration (less than 50% decay) is consistent with strong binding to plasma proteins. In a previous study, Jandorf and McNamara (6) found that plasma radioactivity in rabbits that received  $\text{DF}^{32}\text{P}$  declined at a somewhat greater rate than that reported herein for mice; otherwise, the time courses are reasonably similar. Actually, the plasma time course for  $^3\text{H}$ -DFP in mice is quite similar to that of  $^{32}\text{P}$ -sarin reported by Polak and Cohen (7) for the time period between 10 min and 4 hr. Unfortunately, these investigators did not study earlier time points.

The brain levels of radioactivity, shown in Figure 4, remained rather stable, with only a 20% drop between 5 min and 4 hr. However, the initial drop in brain levels between 1 and 5 min is rather surprising and somewhat difficult to explain. Apparently, the bolus injection results in a large influx of  $^3\text{H}$ -DFP into brain, and a portion of that which is not bound to brain protein rapidly egresses from brain (discussed later). It is important to point out that at these very early times, when plasma levels of  $^3\text{H}$ -DFP are high, radioactivity in blood within the organs could constitute a small portion of the tissue

Figure 3

Time Course of Radioactivity in Plasma After I.V. Injection of  $^3\text{H}$ -DFP.  
Means  $\pm$  S.E. of five mice are presented.

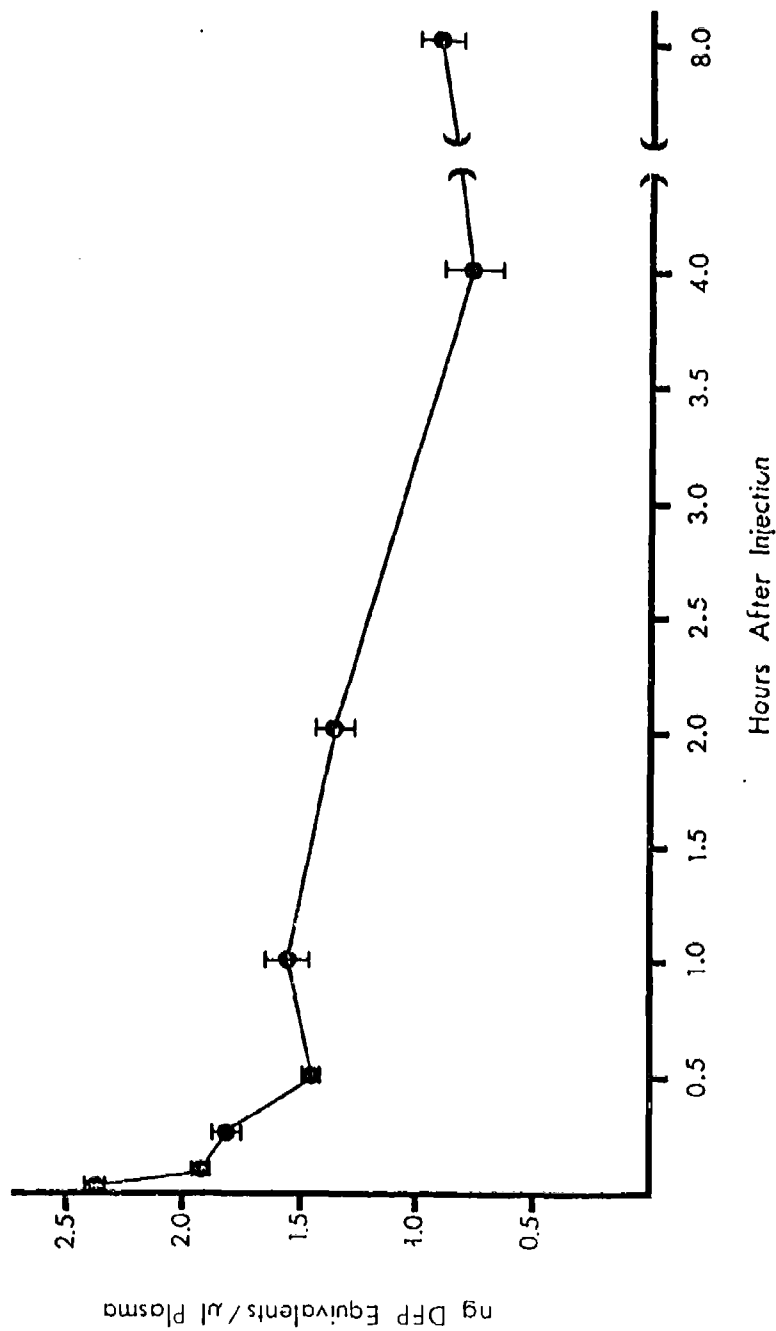
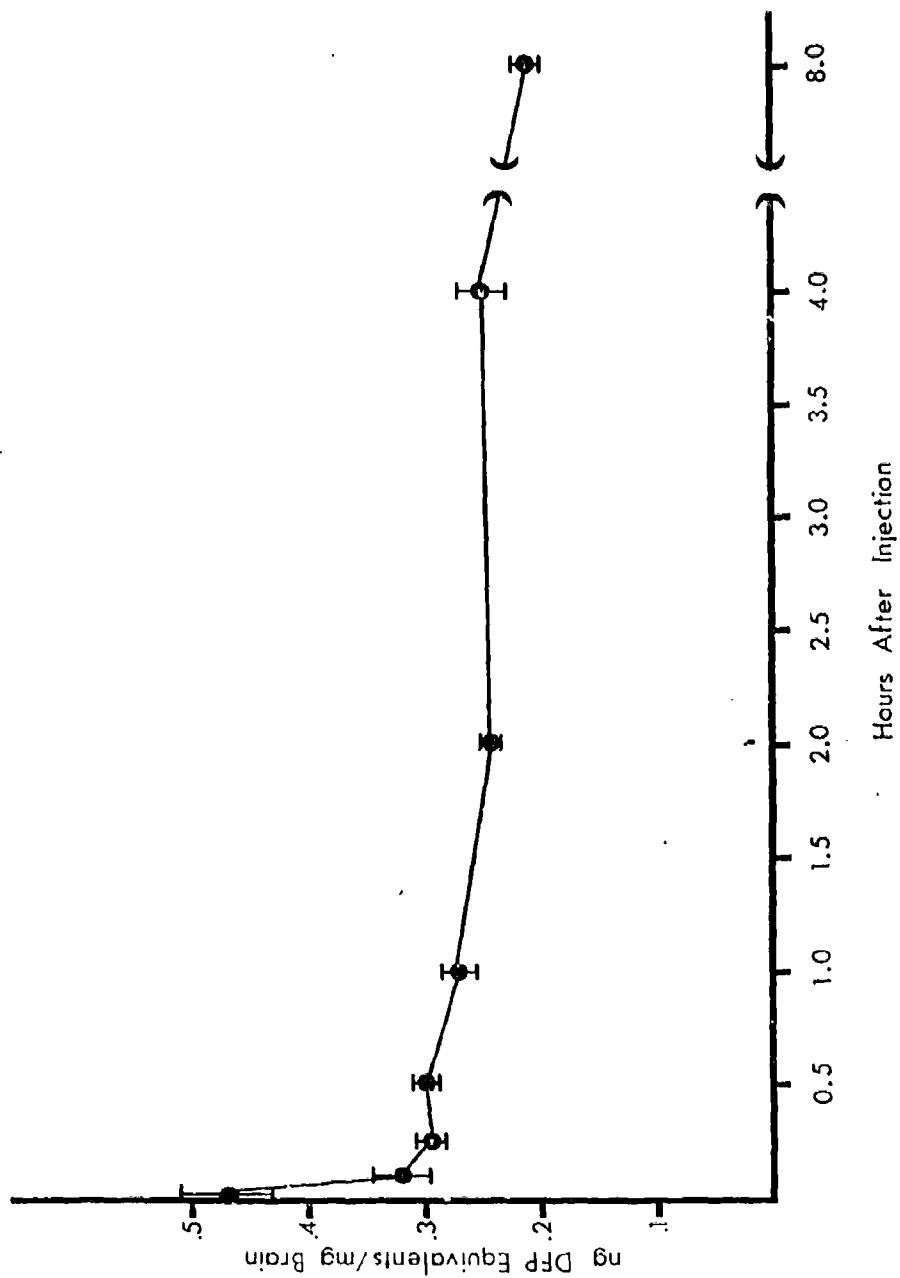


Figure 4

Time Course of Radioactivity in Brain After I.V. Injection of  $^3\text{H}$ -DFP.  
Means  $\pm$  S.E. of five mice are presented.



radioactivity. However, the fall in brain levels of radioactivity is not a result of changes in plasma levels, since plasma levels are stable during this period of time.

The time course of radioactivity in lung, presented in Figure 5, is very similar to that found for brain. The primary difference between lung and brain is the 3-fold higher levels in lung. Jandorf and McNamara (6) have studied the time course of radioactivity in rabbit lung between 10 min and 24 hr after i.v. administration of DF<sup>32</sup>P. They found no change in radioactivity between 10 and 60 min, whereas we find a slight decrease in lung levels in mice. Levels in lungs of both rabbits and mice declined approximately 40-50% by 4 hr (based upon the 10-min values).

The time course of radioactivity in diaphragm, liver and fat of mice are similar in that there is an initial rise in radioactivity between 1 and 5 min (opposite to that found for lung and brain), followed by a gradual decline with time (Figures 6, 7 and 8). The time course in mouse liver differs from that reported for rabbit (6) in that maximum levels in concentrations of radioactivity in rabbit liver were achieved after 60 min as compared to 5 min in mice. Also, rabbit liver dropped 60-70% by 4 hr, whereas the liver levels of radioactivity in mice remained relatively constant during this period of time.

The disposition of radioactivity in diaphragm and heart (Figures 9 and 10) differed from the other tissues somewhat in that there was a more or less even decline in radioactivity during the entire time period investigated.

The organs with the highest concentrations of radioactivity throughout the time course were liver, lung and kidney, followed by diaphragm and heart. The lowest levels were found in fat and brain. Ramachandran (8) studied the disposition of radioactivity in selected tissues of mice at only 30 min after i.v. administration of DF<sup>32</sup>P, and the relative concentrations in kidney, lung and brain were comparable to those reported herein. A major difference in these two studies arises with liver concentrations. Ramachandran (8) found the liver levels to be 8 times higher than the kidney concentrations, whereas we find the liver levels to be only twice as high. Only relative comparisons can be made, since Ramachandran (8) reported data as uncorrected radioactivity.

A comparison of the <sup>3</sup>H-DFP biodisposition to that of other organophosphates suggests differences in the tissue distribution of these agents. <sup>32</sup>P-Sarin has been reported to be present in highest concentrations in kidneys, lungs and plasma with low concentrations in liver (7). A somewhat similar dispositional profile has been reported for <sup>14</sup>C-soman (9). The high concentrations of radioactivity in liver following <sup>3</sup>H-DFP administration could represent an interesting difference in the biodisposition of the organophosphates, provided administered dose and species are not responsible. Apparently, the disposition of soman and sarin in fat has not been investigated. The recent report by Eigenberg et al. (5) revealed that parathion is

Figure 5

Time Course of Radioactivity in Lung After I.V. Administration of  $^3\text{H}$ -DFP.  
Means  $\pm$  S.E. of five mice are presented.

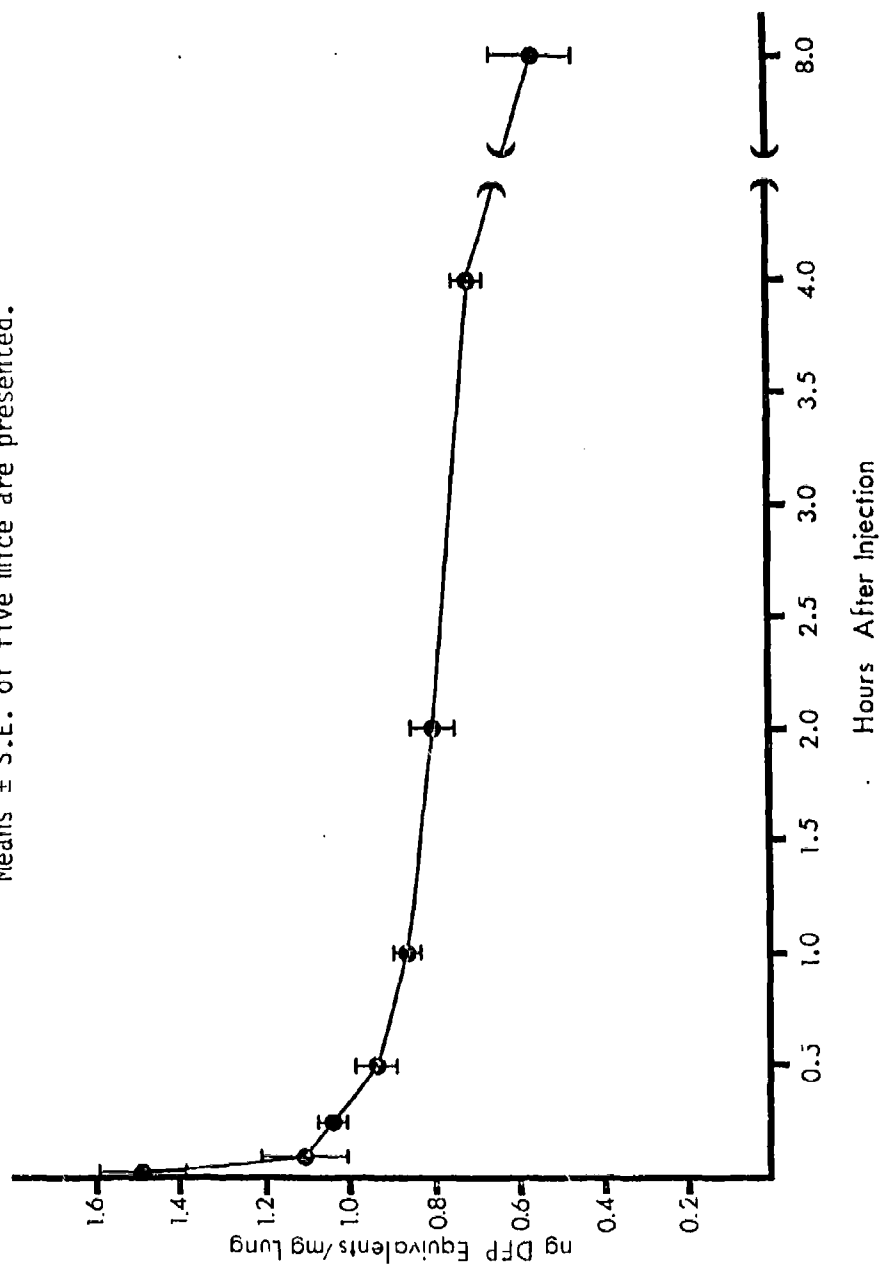


Figure 6

Time Course of Radioactivity in Diaphragm After I.V. Administration of  $^3\text{H}$ -DFP.  
Means  $\pm$  S.E. of five mice are presented.

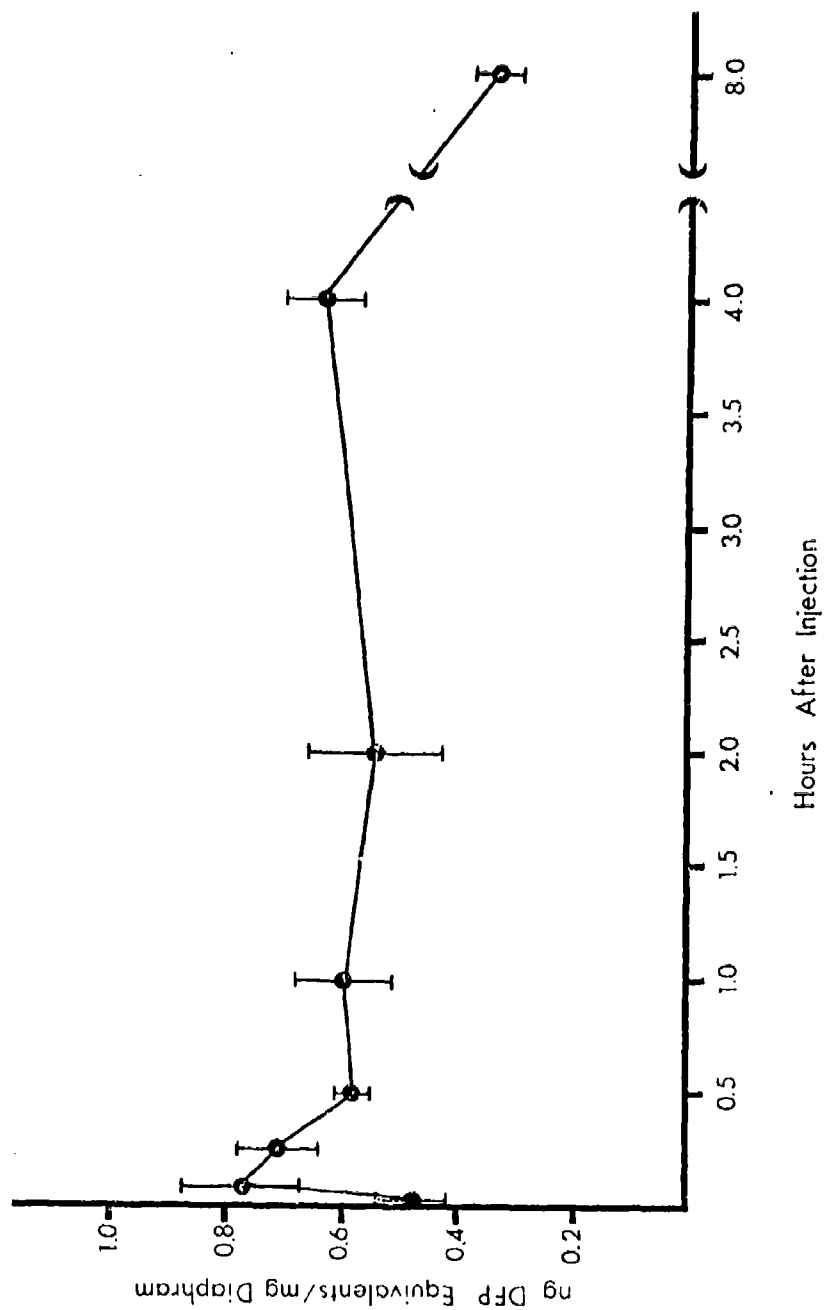


Figure 7

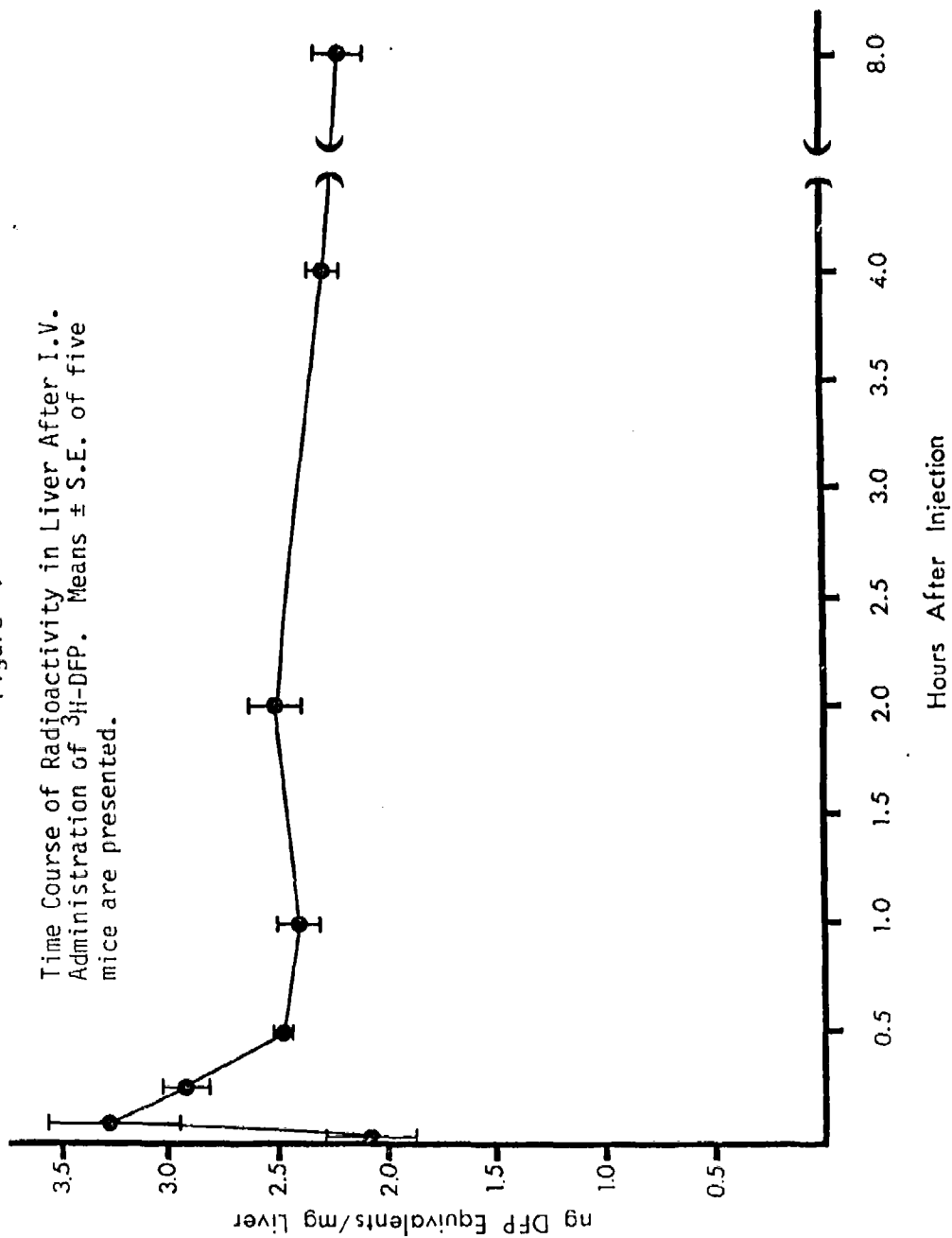


Figure 8

Time Course of Radioactivity in Fat After I.V. Administration of  $^3\text{H}$ -DFP.  
Means  $\pm$  S.E. of five mice are presented.

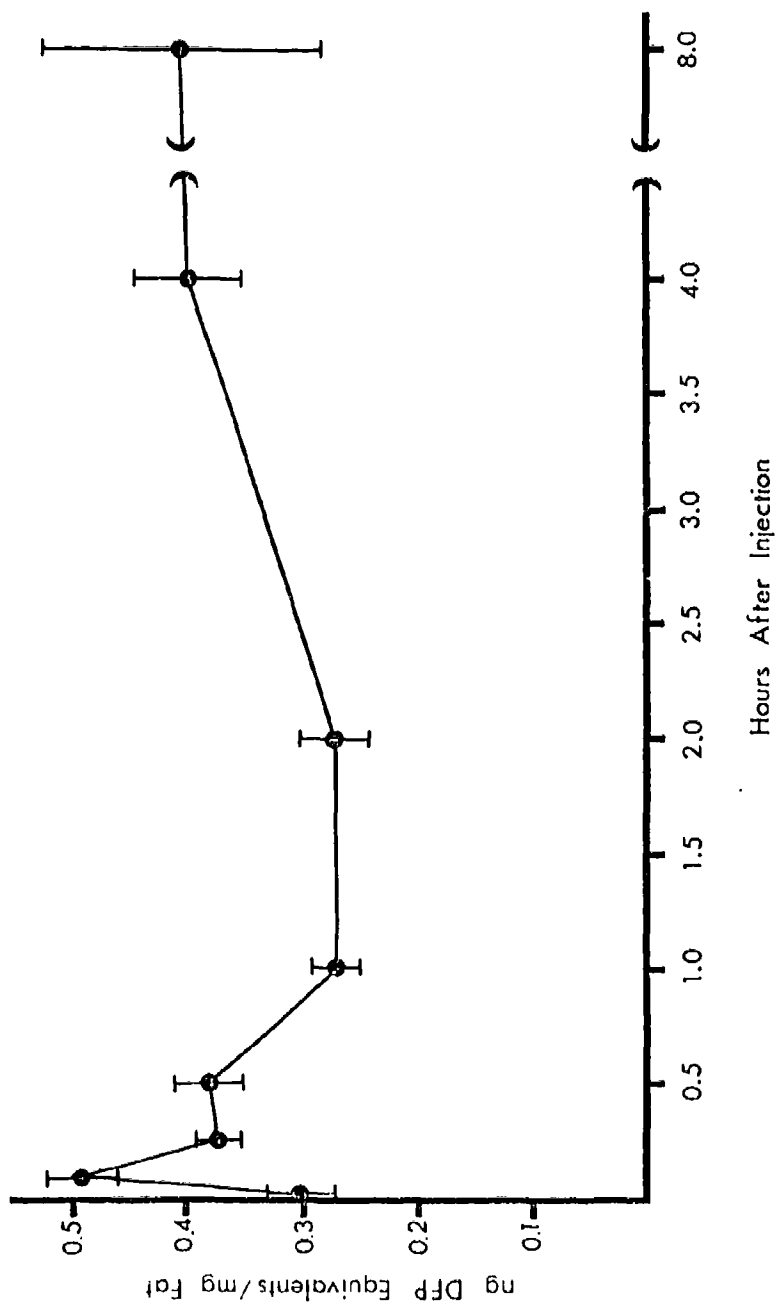


Figure 9

Time Course of Radioactivity in Kidney After I.V. Administration of  $^3\text{H}$ -DFP.  
Means  $\pm$  S.E. of five mice are presented.

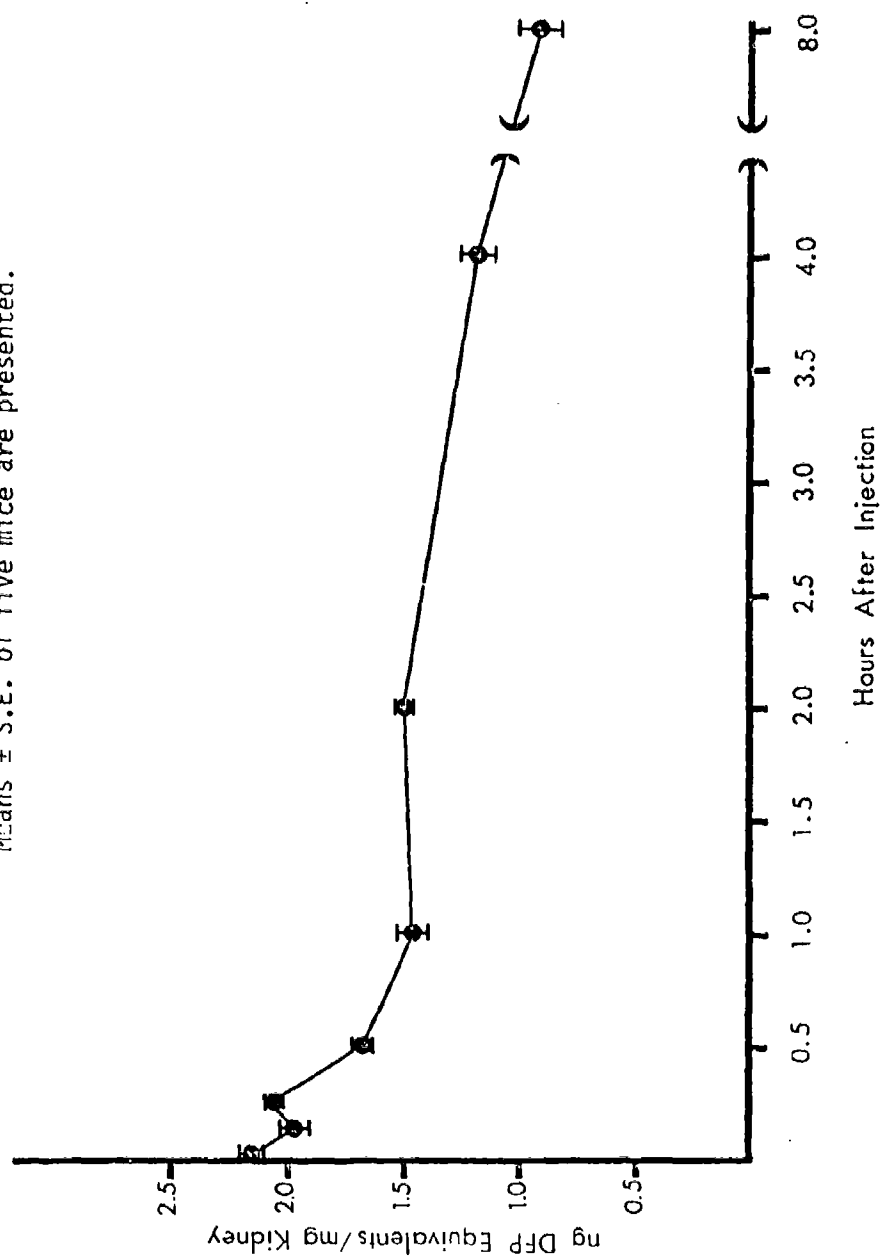
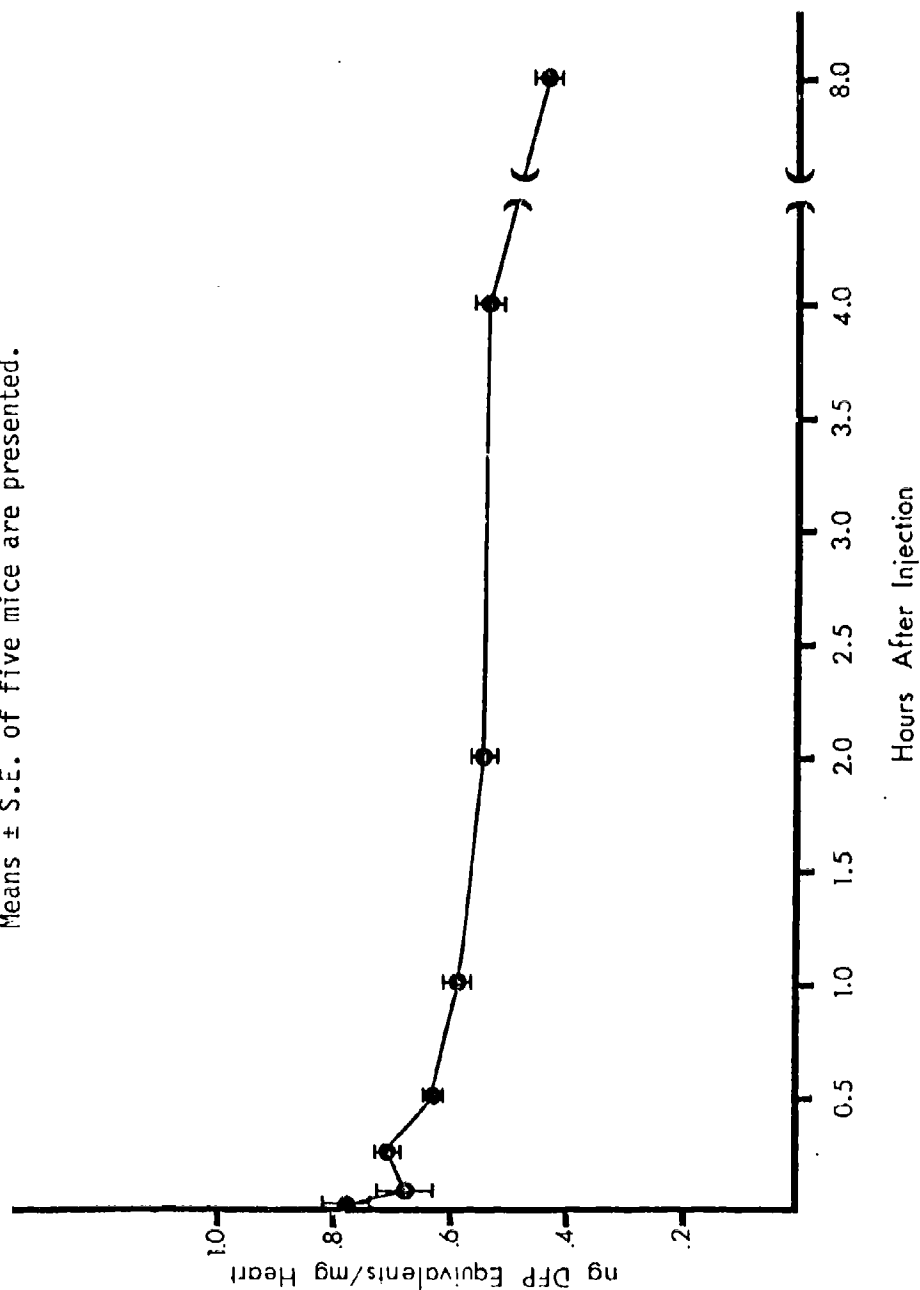


Figure 10

Time Course of Radioactivity in Heart After I.V. Administration of  $^3\text{H}$ -DFP.  
Means  $\pm$  S.E. of five mice are presented.



deposited primarily in fat. It may be that fat deposition is unique for thiophosphates. The deposition of  $^3\text{H}$ -DFP radioactivity was less in fat than in all other tissues except brain.

## 2. Long-term biodisposition of radioactivity.

The disposition of  $^3\text{H}$ -DFP was also investigated at longer periods of time after administration in order to identify potential storage depots. The results are presented in Figures 11-18. The 8-hr time points were replotted on these graphs as a point of reference to the short-term biodisposition. The levels of radioactivity did not decline appreciably between 8 and 24 hr for any tissue with the exception of fat (Figure 16). The major reduction in most tissue levels occurred between 1 and 3 days after administration. The highest levels of radioactivity were in liver and lung after 7 days, and these organs would appear to be likely candidates for storage sites.

A possible complication in the interpretation of the time course of  $^3\text{H}$ -DFP disposition is the fact that a portion of the radiolabeled material may be lost during the so-called "aging process" as depicted in Figure 19. Apparently, the rate of loss of an isopropyl group from DFP bound to esterases in mice has been determined. Berends *et al.* (10) reported that the "aging process" of  $\text{DF}^{32}\text{P}$  bound to horse pseudocholinesterase *in vitro* is approximately 6 hr. It may be that the initial decay of radioactivity that was rapid in some tissues was due in part to this process. However, Berends *et al.* (10) found only 12% of the  $^{32}\text{P}$  bound to horse pseudocholinesterase was present as monoisopropylphosphate after 1 hr. Cohen and Warringa (11) reported that 80% of  $\text{DF}^{32}\text{P}$  in the human body was excreted as diisopropylphosphoric acid and 20% remained bound to tissues.

If the rate of aging is similar in mice, then the decline in radioactivity that occurred within the first hour after administration of  $^3\text{H}$ -DFP would be due to excretion of  $^3\text{H}$ -DFP rather than loss of  $^3\text{H}$ -isopropanol. Comparison of the  $^3\text{H}$ -DFP data reported herein with previous studies of  $\text{DF}^{32}\text{P}$  is difficult because previously only a few tissues have been studied at selected time points. The time course of  $\text{DF}^{32}\text{P}$  binding in kidney and liver of rabbits is reasonably similar to that of  $^3\text{H}$ -DFP in mouse kidney and liver. This issue will be clarified by examining some of the tissue homogenates for the presence of  $^3\text{H}$ -monoisopropylphosphate at a few time points. The procedure outlined by Berends *et al.* (10) will be followed. These data will also establish the rapidity with which aging occurs in the mouse.

## 3. Extraction of unbound radioactivity from tissue homogenates.

Data on the radioactivity removed by ethyl extraction are presented in Table 4. One min after i.v. administration of  $^3\text{H}$ -DFP (1 mg/kg), 55% of the radioactivity in brain is extracted with ethyl acetate and the remainder is bound.

Figure 11

Long-Term Time Course of Radioactivity in Plasma After I.V. Administration of  $^3\text{H}$ -DFP. Means  $\pm$  S.E. of five mice are presented.

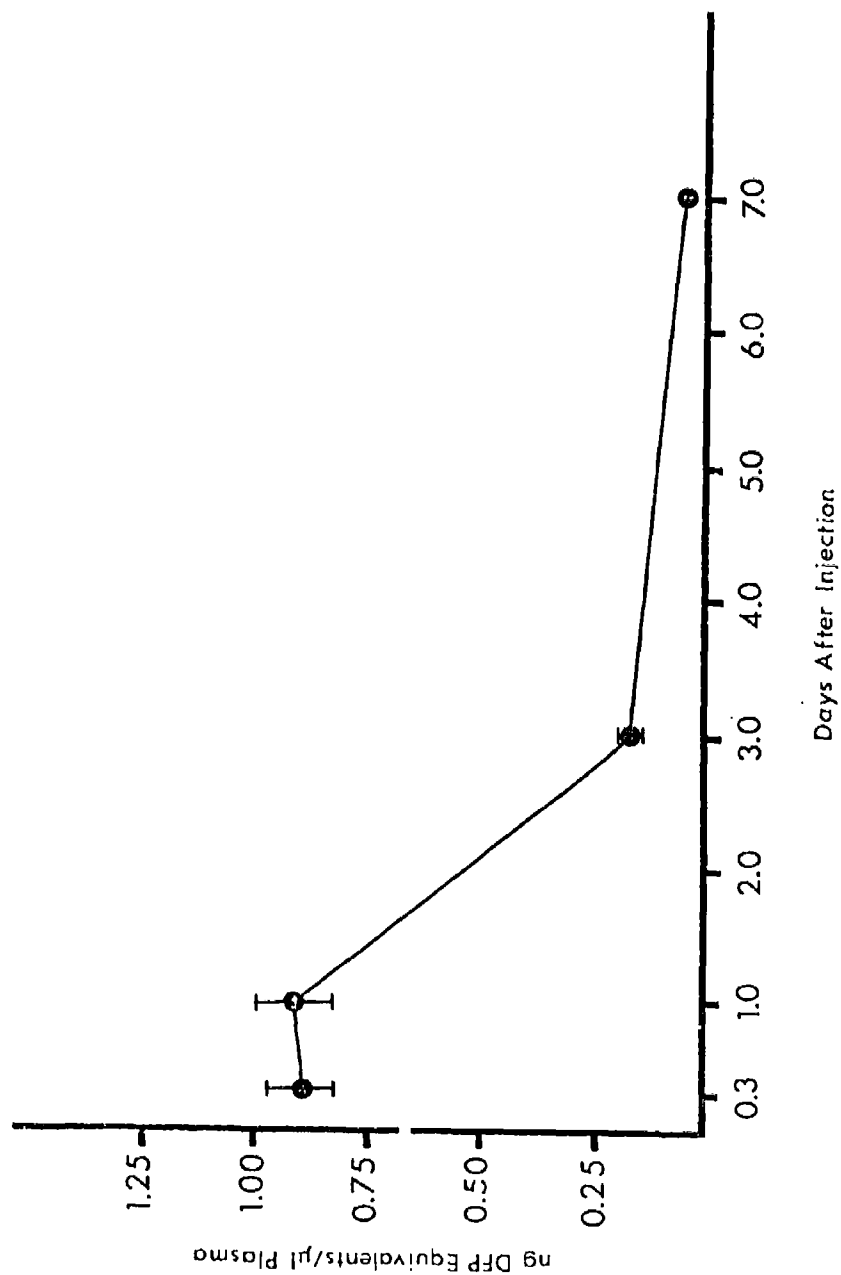


Figure 12

Long-Term Time Course of Radioactivity in Brain After I.V. Administration of  $^3\text{H}$ -DTP. Means  $\pm$  S.E. of five mice are presented.

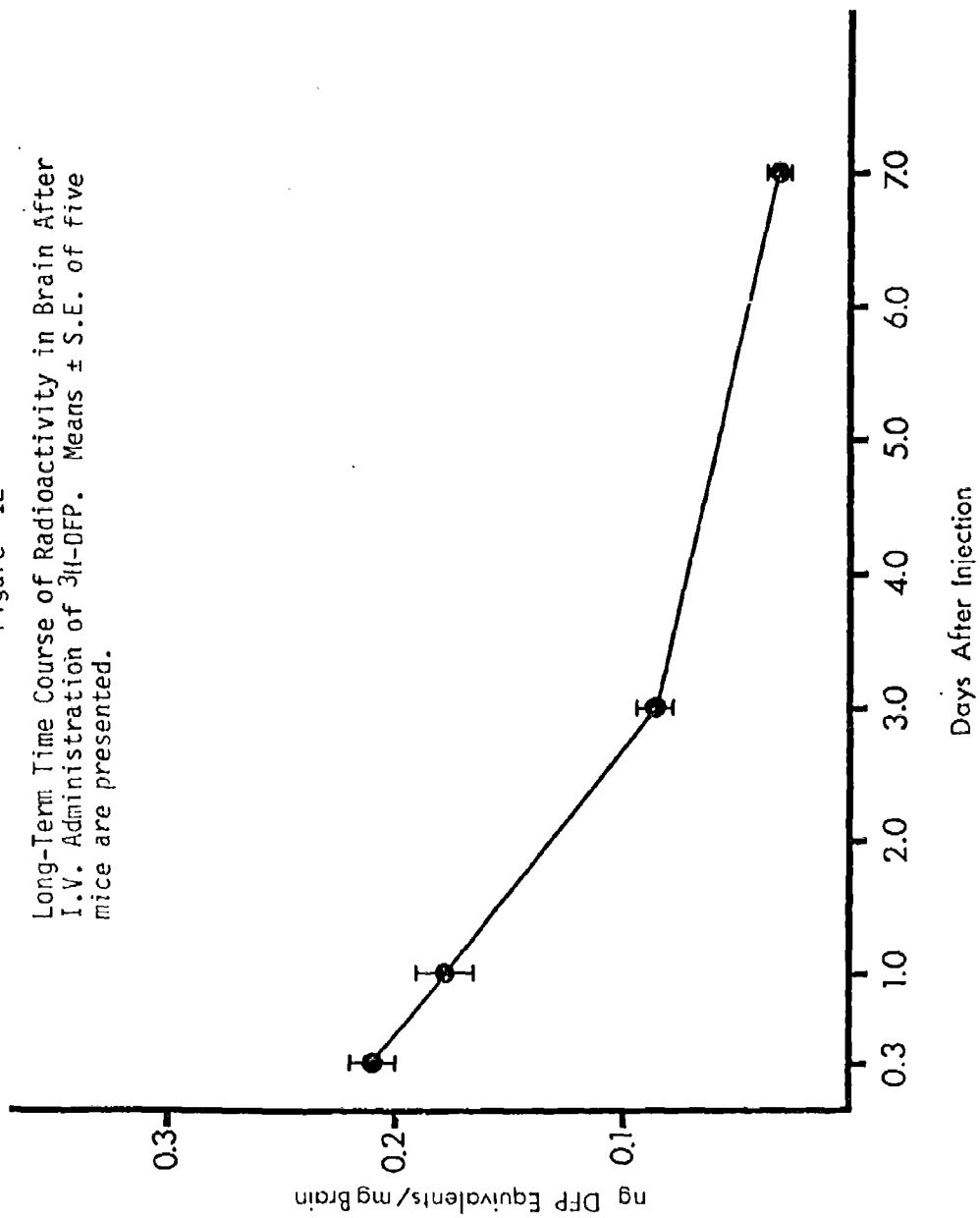


Figure 13

Long-Term Time Course of Radioactivity in Lung After I.V. Administration of  $^3\text{H}$ -DFP. Means  $\pm$  S.E. of five mice are presented.

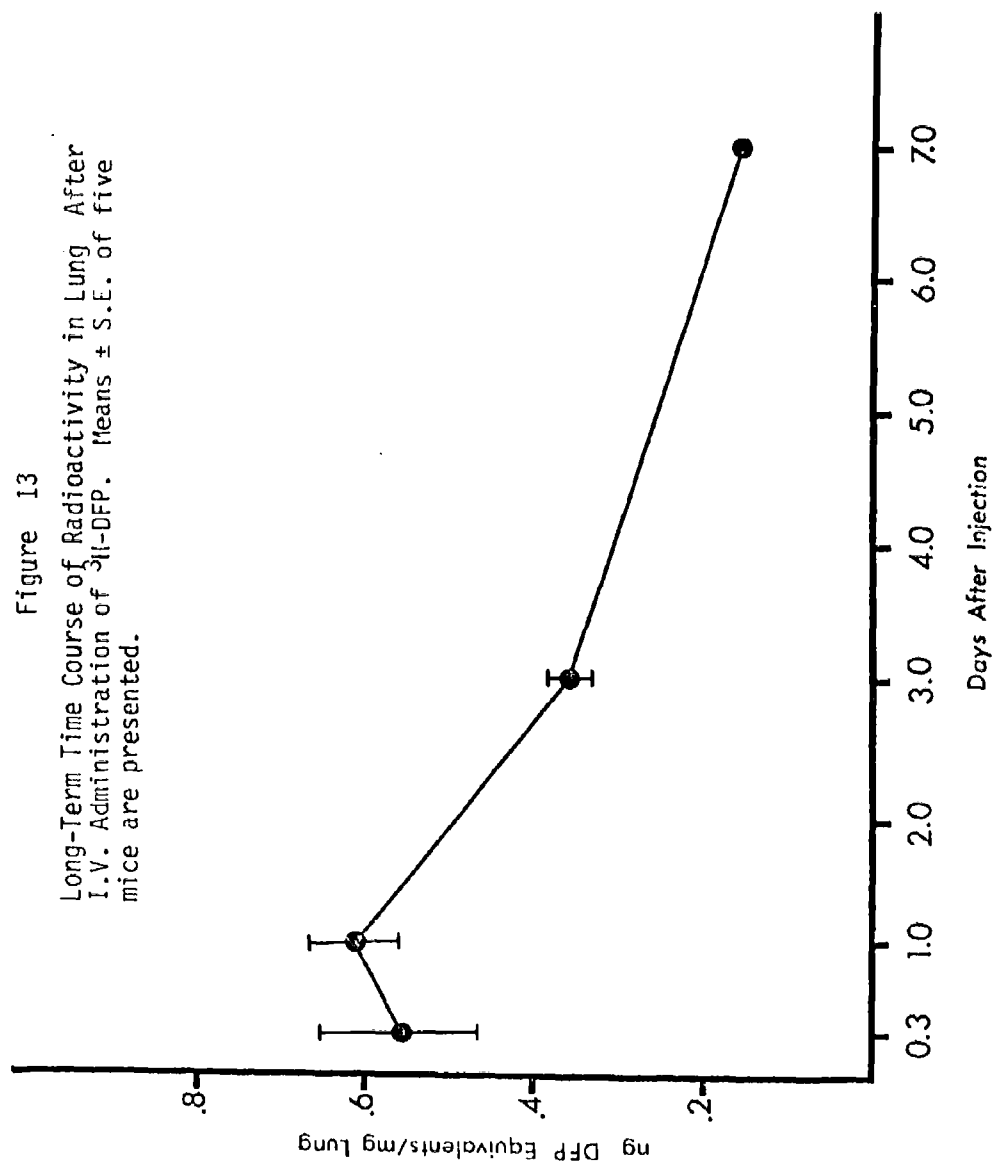


Figure 14

Long-Term Time Course of Radioactivity in Diaphragm After I.V. Administration of  $^3\text{H}$ -DFP. Means  $\pm$  S.E. of five mice are presented.

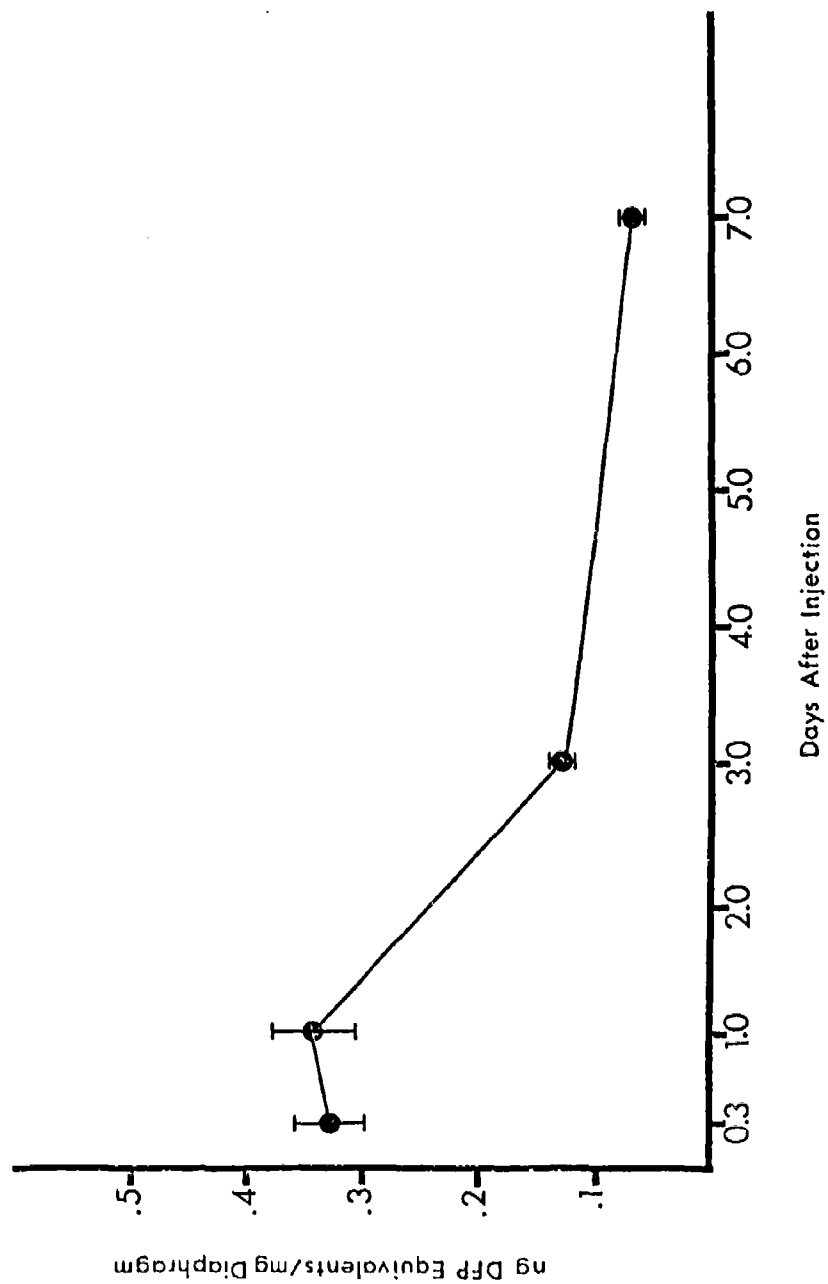


Figure 15

Long-Term Time Course of Radioactivity in Liver After I.V. Administration of  $^{311}\text{I}$ -DTP. Means  $\pm$  S.E. of five mice are presented.

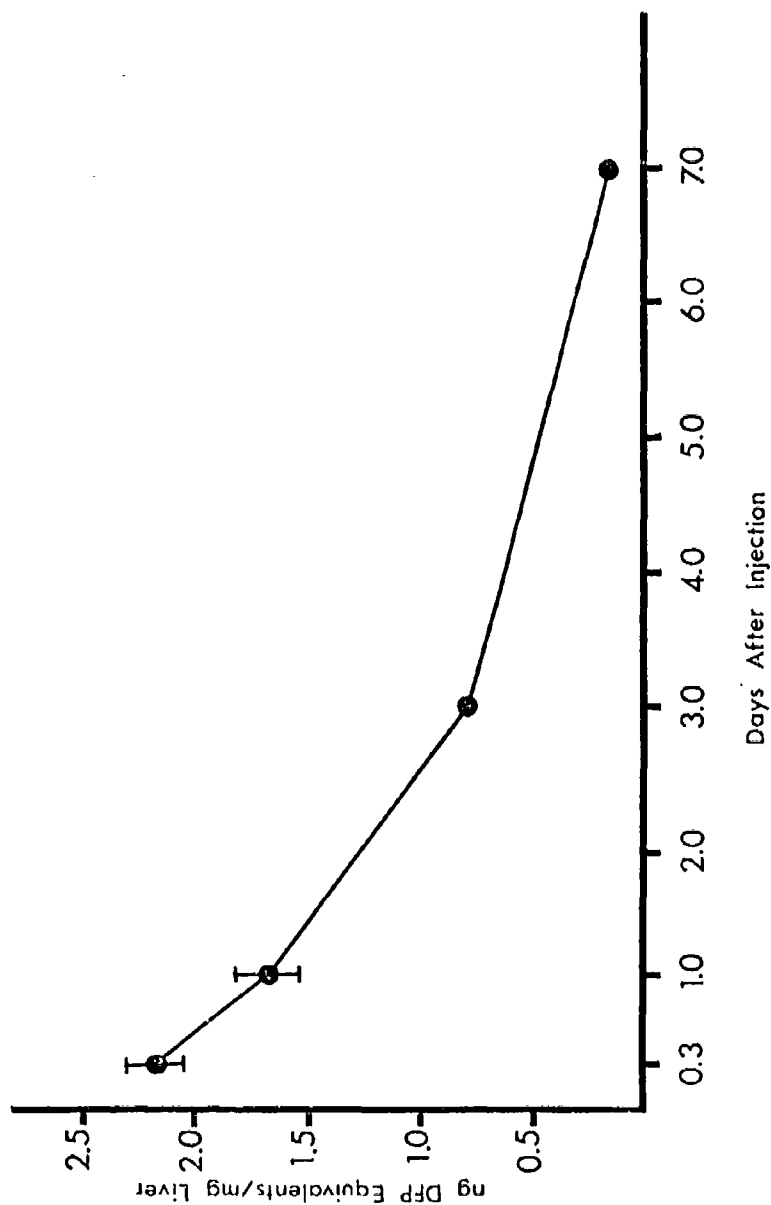


Figure 16

Long-Term Time Course of Radioactivity in Fat After I.V. Administration of  $^3\text{H}$ -DFP. Means  $\pm$  S.E. of five mice are presented.

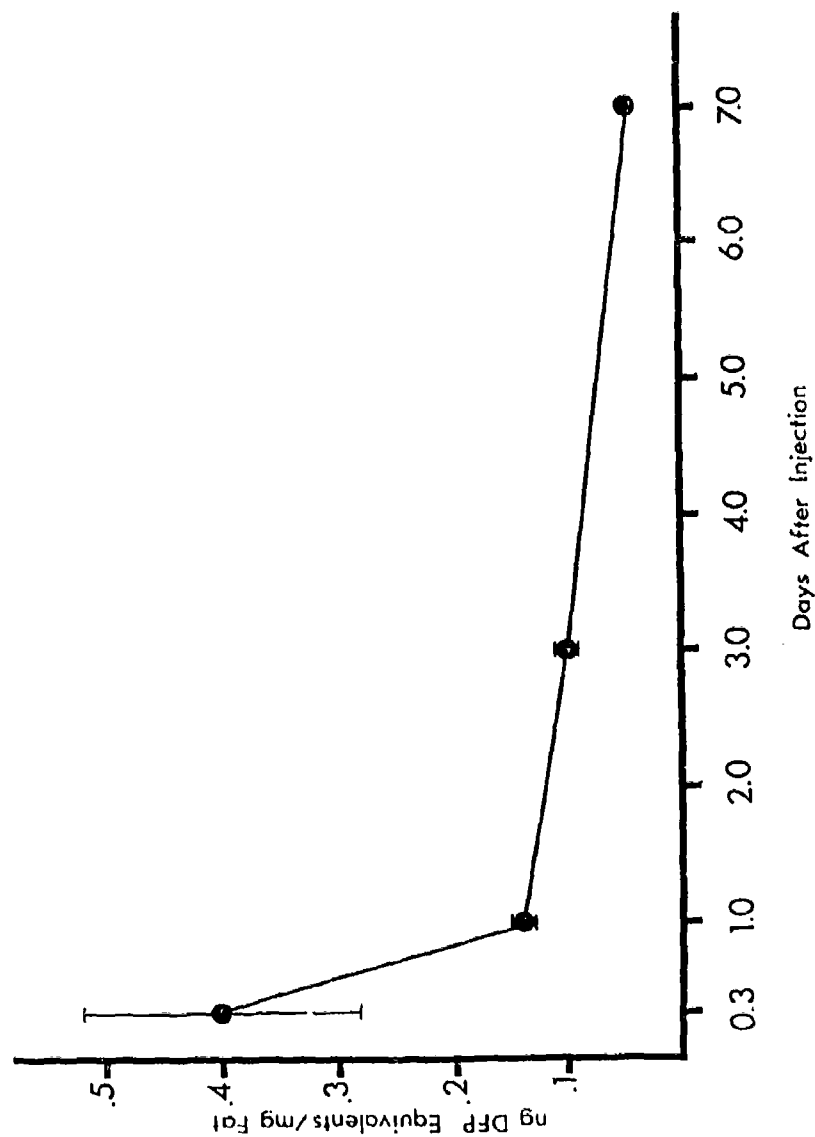


Figure 17

Long-Term Time Course of Radiogactivity in Kidney  
After I.V. Administration of  $^3\text{H}$ -DTP. Means  $\pm$  S.E.  
of five mice are presented.

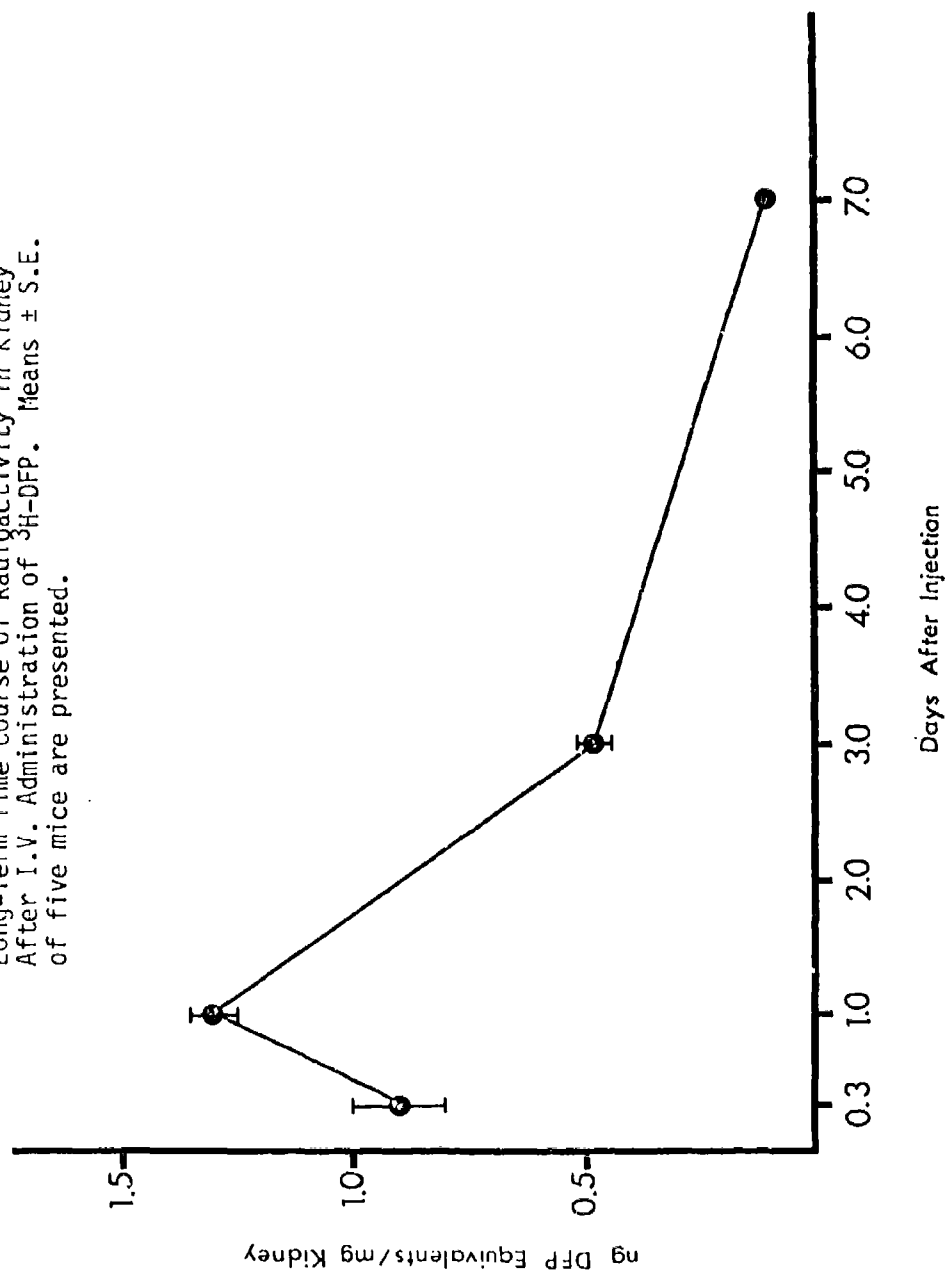


Figure 18

Long-Term Time Course of Radioactivity in Heart  
After I.V. Administration of  $^3\text{H}$ -DFP. Means  $\pm$  S.E.  
of five mice are presented.

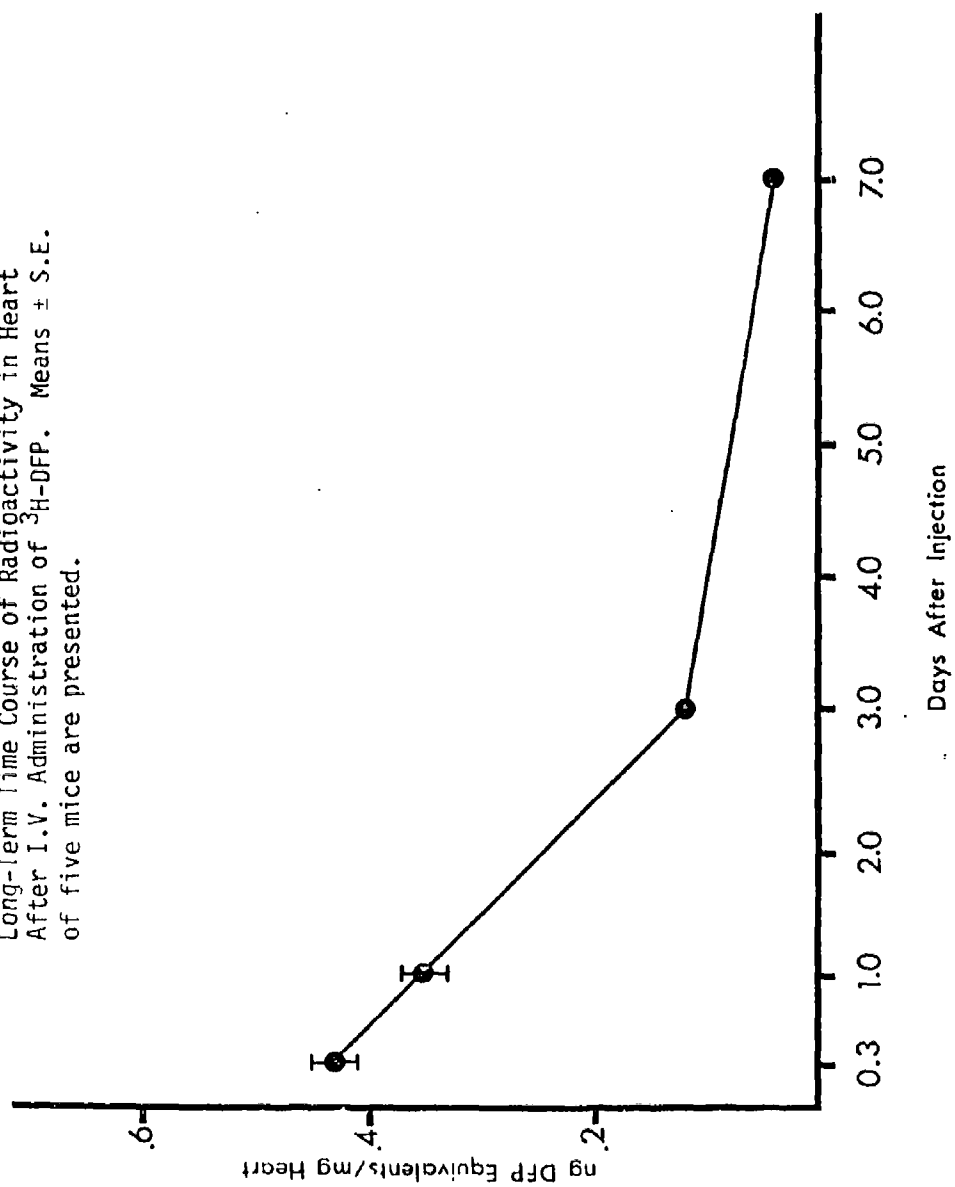
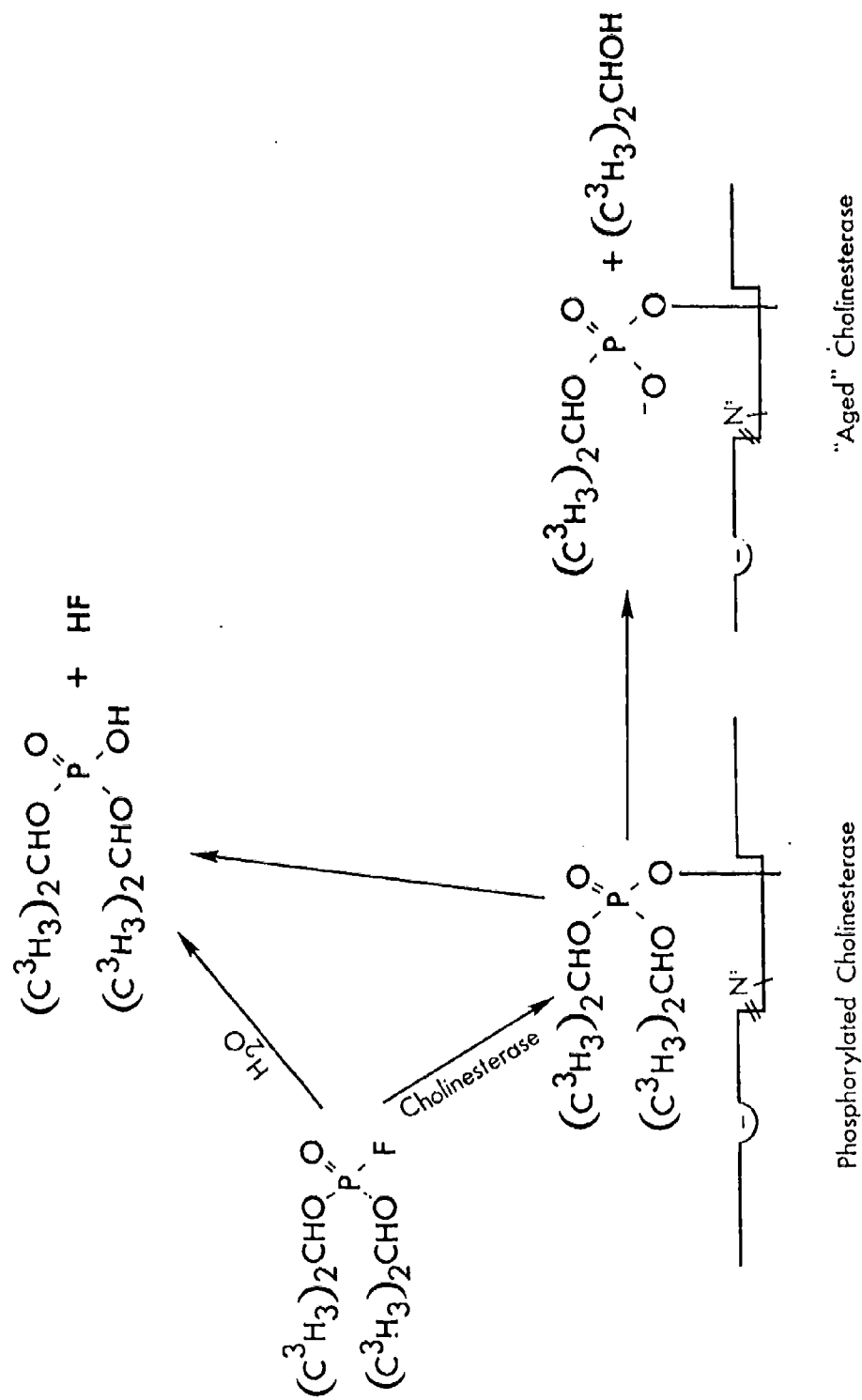


Figure 19  
FATE OF DFP IN VIVO



This rather high percentage of unbound radioactivity is consistent with our previous suggestion that the initial rapid fall in brain levels is due to egress of unbound radioactivity. It is doubtful that appreciable aging would have occurred by 1 min. By 30 min, only 24% of the brain radioactivity is extracted with ethyl acetate and after 4 hrs 9% is extracted. A relatively small proportion (13-32%) of the radioactivity in the other tissues is extracted by ethyl acetate at the 1-min time point; the extractable radioactivity decreases further with time. Very little of the radioactivity in plasma is removed by ethyl acetate at any time. If appreciable aging were occurring, one would expect higher levels of radioactivity in the form of  $^3\text{H}$ -isopropanol in plasma. A future objective will be to identify the material that is removed by ethyl acetate extraction.

TABLE 4

Extraction of Tissue Radioactivity from Animals Treated with  $^3\text{H}$ -DFP

Tissue	% Extracted (Mean $\pm$ S.E., N=5)			
	1 Min	30 Min	4 Hrs	3 Days
Brain	55 $\pm$ 1	24 $\pm$ 3	9 $\pm$ 1	7 $\pm$ 1
Liver	13 $\pm$ 2	6 $\pm$ 0	1 $\pm$ 0	1 $\pm$ 0
Diaphragm	20 $\pm$ 5	8 $\pm$ 1	5 $\pm$ 1	3 $\pm$ 0
Lung	16 $\pm$ 4	8 $\pm$ 0	3 $\pm$ 0	2 $\pm$ 0
Heart	24 $\pm$ 2	11 $\pm$ 0	4 $\pm$ 0	4 $\pm$ 0
Fat	16 $\pm$ 5	15 $\pm$ 1	2 $\pm$ 0	2 $\pm$ 0
Plasma	3 $\pm$ 0	4 $\pm$ 0	3 $\pm$ 0	5 $\pm$ 1

### C. Conclusions

$^3\text{H}$ -DFP is rapidly distributed to tissues after i.v. administration, and the levels of radioactivity remain high for up to 24 hr after administration. The tissue levels of radioactivity do not decline appreciably until 3 days following acute administration, which is consistent with irreversible or nearly irreversible binding to protein.  $^3\text{H}$ -DFP would accumulate in most organs of the body if animals were chronically exposed. However, acute or occasional exposure would likely result in disposition primarily in lung and liver.

At early times after exposure to  $^3\text{H}$ -DFP, a major portion of the radioactivity in the brain was extracted by ethyl acetate and presumed to be unbound  $^3\text{H}$ -DFP. Most of the radioactivity in the other tissues was already tightly bound within a few min after drug administration. The percentage of radioactivity that could be extracted with ethyl acetate declined in all tissues with time.

The nature of the free and bound radioactivity remains to be established. It will be important to establish the time required for the  $^3\text{H}$ -DFP-enzyme complex to age for comparison with the other organophosphates and for correcting for any loss of the radiolabel. The present data do not indicate a significant amount of aging unless it occurs extremely slowly. These results are too preliminary to draw conclusions about specific patterns of DFP accumulation and enzyme aging. Further studies are needed to determine whether these speculations are correct.

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